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BIOASSAY REPORT

Acute toxicity of Dowtherm A to fishes and aquatic invertebrates.

Bionomics, Inc.
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These investigations were performed at the aquatic toxicology laboratory of Bionomics, Inc., Wareham, Massachusetts. The susceptibility of the organisms to the test chemical was measured in terms of the median tolerance limit (TL_{50}), the nominal concentration of the chemical in water which causes 50 percent response under the test conditions during a specified time interval. The response measured in all tests was death, except for the test with Daphnia where immobilization was the measured response. The prediction of the TL_{50} value and its 95% confidence interval, was based on conversion of the concentrations tested and the corresponding observed percent mortalities to logs and probits respectively, and the subsequent mathematical calculation of a linear regression equation. The toxicity of Dowtherm A to those invertebrates tested under static bioassay conditions is expressed as a 48-hour TL_{50} . The toxicity of the chemical to those species tested under dynamic bioassay conditions is expressed in terms of the incipient or time-independent TL_{50} (i. e. that concentration which produces 50% mortality in a bioassay during which no additional mortality was observed during the last 48 hours of the test period).

The aquatic invertebrates tested under static bioassay conditions were scud (Gammarus fasciatus), water flea (Daphnia magna), shrimp (Palaemonetes vulgaris), damsel fly nymph (Ischnura sp.), the dragon fly nymph (Libellulidae sp.), and the caddis fly larva (Hydropsychidae sp.). The water flea was cultured in the laboratory from a stock acquired from the Federal Water Quality Administration (now Environmental Protection Agency) in Cincinnati, Ohio. The shrimp were provided by the Marine Biological Laboratory at Woods Hole, Massachusetts; the remaining

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aquatic invertebrates tested under static conditions were acquired from a local commercial biological supply house. Static bioassays were conducted without aeration and with one introduction of Dowtherm A at the beginning of the test. Tests with waterfleas were conducted in glass beakers containing 150 mls of test diluent. All other static tests were conducted in 1.5 gallon aquaria containing 4 liters of water. In each test ten early instar animals were placed in each test container and held for a six-hour acclimation period before the test chemical was added. All static tests were conducted at 18° (± 0.5).

Dynamic bioassays were conducted using crayfish (Procambarus clarkii); two estuarine fishes, killifish (Fundulus heteroclitus) and stickleback (Gasterosteus aculeatus); and four freshwater fishes, bluegill (Lepomis macrochirus), channel catfish (Ictalurus punctatus), fathead minnow (Pimephales promelas), and rainbow trout (Salmo gairdneri). The crayfish were obtained from a commercial producer in northern Louisiana; the estuarine fish species from the Marine Biological Laboratory in Woods Hole, Massachusetts. The catfish and minnows were obtained from a commercial grower in Arkansas, the bluegill from a similar source in Connecticut, and the rainbow trout from a commercial producer in Wareham, Massachusetts. The test procedures for the dynamic bioassays except for conditions described below, are in complete accordance with the Fish Bioassay Procedure described in the 1970 edition of Standard Methods (APHA). Dynamic tests were conducted in a continuous-flow proportional dilution apparatus (Mount and Brungs, 1967)¹, which

¹ Mount, D. I. and W. A. Brungs, A Simplified Dosing Apparatus for Fish Toxicology Studies. Water Research 1: 21 (1967).

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provides for the automatic intermittent introduction of the test material and diluent water to a control unit. Flow rate to each 30-liter aquaria was 5 l/hour. Fish were conditioned to the test system for 96 hours prior to the introduction of the chemical. Ten specimens were tested in each concentration.

All tests were conducted in aerated well water having a pH of 7.1, total hardness as CaCO_3 of 25 mg/l, and total alkalinity as CaCO_3 of 47 mg/l. Test solutions for static tests were prepared prior to beginning the tests by adding appropriate amounts of chemicals dissolved in acetone to the test diluent. A similar procedure was used in dynamic tests to initially establish the desired concentration; then the continuous-flow systems were utilized to maintain the desired test concentrations. In each test the control groups were exposed to the highest concentration of the solvent to which specimens in the test solutions were exposed. Mortality was recorded every 24 hours during the test period. In both static and dynamic tests a minimum of seven chemical concentrations were tested.

The results of the bioassays with Dowtherm A are summarized in Table 1. A very distinct mortality syndrome was observed with all species of fishes. In every case, those fish effected by the chemical lost equilibrium and were observed to be lying on the bottom for as long as 72-96 hours before succumbing to the toxicant. No such distinct behavior was observed with the invertebrates tested. The relative differences in susceptibility of waterfleas and scud to Dowtherm

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is not surprising since data from the literature indicated that these species are generally among the most sensitive aquatic forms.

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Table 1- Acute toxicity of Dowtherm A to aquatic organisms. The data is based on the results of bioassays^a conducted at the aquatic toxicology laboratory of Bionomics, Inc. in Wareham, Massachusetts.

Species Common name)	Bioassay (Type)	Mean Weight (g)	TL ₅₀ (95% confidence interval) ^b (µg/l)	No effect ^c level	Duration (hrs)
<u>Salmo gairdneri</u> (rainbow trout)	Dynamic	7.9	2720(1990-3720)	1600	120
<u>Lepomis macrochirus</u> (bluegill)	"	0.8	2110(1080-2650)	1100	120
<u>Ictalurus punctatus</u> (channel catfish)	"	4.7	2350(1760-3150)	1800	120
<u>Pimephales promelas</u> (fathead minnows)	"	1.9	3170(2350-4260)	1800	144
<u>Fundulus heteroclitus</u> (killifish)	"	9.0	2700(2220-3290)	1800	144
<u>Gasterosteus aculeatus</u> (stickleback)	"	0.8	1390(1960-2000)	700	120
<u>Procambarus clarkii</u> (crayfish)	"	30.0	2400(1600-3200)	1600	120
<u>Palaemonetes vulgaris</u> (grass shrimp)	Static	—	1600(1200-2100)	1200	48
<u>Daphnia magna</u> (waterflea)	"	—	120 (30-430)	50	48
<u>Gammarus fasciatus</u> (scud)	"	—	40 (20-50)	20	48
<u>Libellulidae</u> sp. (caddis fly larva)	"	—	2600(1000-4200)	1400	48
<u>Hydropsychidae</u> sp. (dragon fly nymph)	"	—	4700(3900-5800)	3200	48
<u>Ischnura</u> sp. (damselfly nymph)	"	—	3900(3100-4900)	2100	48

^a All tests were conducted at 18°C (± 0.5) except for trout which were tested at 13°C (± 0.5).

^b For static tests the 48-hour TL₅₀ value is given; for the dynamic tests the incipient LC₅₀ value is given.

^c No effect level is that concentration at which no apparent signs of stress were observed (e. g. no hyperactivity or loss of equilibrium).

^d Shrimp were tested in sythetic sea water having a salinity of 24 parts per thousand.